AFRL-RX-WP-JA-2018-0078

GENOME OF A BYSSOCHLAMYS SP. STRAIN ISOLATED FROM FOULED B20 BIODIESEL (POSTPRINT)

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18 January 2018
Interim Report

(STINFO COPY)

AIR FORCE RESEARCH LABORATORY
MATERIALS AND MANUFACTURING DIRECTORATE
WRIGHT-PATTERSON AIR FORCE BASE, OH 45433-7750
AIR FORCE MATERIEL COMMAND
UNITED STATES AIR FORCE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
18 January 2018	Interim	8 September 2014 – 18 December 2017

4. TITLE AND SUBTITLE
GENOME OF A BYSSOCHLAMYS SP. STRAIN ISOLATED FROM FOULED B20 BIODIESEL (POSTPRINT)

5a. CONTRACT NUMBER
FA8650-15-D-5405-0002

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
62102F

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(continued on page 2)

5d. PROJECT NUMBER
4348

5e. TASK NUMBER 0002

5f. WORK UNIT NUMBER
X0YE

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Air Force Research Laboratory
Materials and Manufacturing Directorate
Wright-Patterson Air Force Base, OH 45433-7750
Air Force Materiel Command
United States Air Force

10. SPONSORING/MONITORING AGENCY ACRONYM(S)
AFRL/RXAS

11. SPONSORING/MONITORING AGENCY REPORT NUMBER(S)
AFRL-RX-WP-JA-2018-0078

12. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution Statement A. Approved for public release: distribution unlimited.

13. SUPPLEMENTARY NOTES
PA Case Number: 88ABW-2018-0191; Clearance Date: 18 Jan 2018. This document contains color. Journal article published in Genome Announcements, Vol. 6, No. 9, Mar 2017. © 2017 American Society for Microbiology. The U.S. Government is joint author of the work and has the right to use, modify, reproduce, release, perform, display, or disclose the work.
The final publication is available at https://genomea.asm.org doi: 10.1128/genomeA.00085-18

14. ABSTRACT *(Maximum 200 words)*
Byssochlamys sp. strain AF001 is a filamentous fungus isolated from fouled B20 biodiesel. Its growth on B20 biodiesel results in the degradation and fouling of the fuel and higher rates of corrosion in affected storage tanks. The genome of *Byssochlamys* sp. AF001 is 35.9 Mbp and is composed of 10 scaffolds, with a G+C content of 45.89%.

15. SUBJECT TERMS
Biodiesel; diesel; fossil fuel; microbial degradation; B20; fungal fuel contaminant; Hormoconis resinae; Byssochlamys

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT:	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON (Monitor)
a. REPORT	b. ABSTRACT	c. THIS PAGE			Lawrence Brott
Unclassified	Unclassified	Unclassified	SAR	5	19b. TELEPHONE NUMBER *(Include Area Code)* (937) 255-9157

REPORT DOCUMENTATION PAGE Cont'd

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EUKARYOTES

Genome Sequence of a *Byssochlamys* sp. Strain Isolated from Fouled B20 Biodiesel

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ABSTRACT *Byssochlamys* sp. strain AF001 is a filamentous fungus isolated from fouled B20 biodiesel. Its growth on B20 biodiesel results in the degradation and fouling of the fuel and higher rates of corrosion in affected storage tanks. The genome of *Byssochlamys* sp. AF001 is 35.9 Mbp and is composed of 10 scaffolds, with a G+C content of 45.89%.

Biodiesel is a widely used additive in ultralow-sulfur diesel (ULSD), both to improve the lubricating properties of the fuel and to reduce our reliance on fossil fuels (1). A 20% mixture of ULSD and biodiesel, B20, is commonly used across the United States. Unfortunately, biodiesel is more susceptible to degradation by microorganisms than is neat diesel (diesel without biodiesel added) (2). Studies into the microbial degradation of B20 have focused on either bacterial consortia or a common fungal fuel contaminant, *Hormoconis resinae* (3, 4). It is important to industry and end-users of biodiesel fuel blends that we expand our knowledge of other microorganisms capable of fuel degradation (5). A member of the filamentous fungal genus *Byssochlamys* (order Eurotiales; here referred to as *Byssochlamys* sp. AF001) was isolated from fouled B20 biodiesel in a storage tank on a U.S. Air Force base. The genome of *Byssochlamys* sp. AF001 was sequenced as a means to better understand its ability to grow on B20 as a sole carbon and energy source, to cause fouling and degradation of fuels, and to accelerate microbiologically influenced corrosion of carbon steel.

Genomic DNA was extracted from a pure culture of *Byssochlamys* sp. AF001 using the Xpedition soil/fecal DNA miniprep kit (Zymo Research Corp., Irvine, CA) for sequencing on the Illumina MiSeq platform using PE250 sequencing chemistry. Genomic DNA was also extracted for long-read sequencing to improve the assembly using an optimized phenol-chloroform-isoamyl alcohol method (6) and sequenced on an Oxford Nanopore MinION sequencer using two R9 flow cells. Assembly was carried out with the SPAdes assembler version 3.11.1 (7), using k-mer values ranging from 21 to 127. Genome assembly statistics were computed using QUAST (8). An annotation was then produced using the GenSAS pipeline (https://www.gensas.org/) and GhostKOALA (9).

The *Byssochlamys* sp. AF001 assembly was composed of 10 scaffolded contiguous sequences. The N_{50} was 4.6 Mbp, the total genome length was 35.9 Mbp, and the G+C content was 45.89%. A putative esterase/lipase, potentially able to cleave the fatty acid-methyl ester (FAME) to its constituent fatty acid and methanol, was located on scaffold 4. Analysis by GhostKOALA suggests that *Byssochlamys* sp. AF001 is capable of metabolizing methanol during the degradation of FAME found in biodiesel, similar to other fungi (10).

Received 23 January 2018 **Accepted** 6 February 2018 **Published** 1 March 2018

Citation Stamps BW, Andrade OC, Lyon WJ, Floyd JG, Nunn HS, Bojanowski CL, Crookes-Goodson WJ, Stevenson BS. 2018. Genome sequence of a *Byssochlamys* sp. strain isolated from fouled B20 biodiesel. Genome Announc 6:e00085-18. https://doi.org/10.1128/genomeA.00085-18.

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The genome described here is of particular interest to further studies of biodiesel degradation both in field and laboratory experiments. Future work will leverage this genome sequence and others to better understand the underlying molecular mechanisms of biodiesel degradation.

Accession number(s). This whole-genome shotgun sequencing project has been deposited at GenBank under the accession number PNEM00000000.

ACKNOWLEDGMENTS

The research reported in this publication has been cleared for public release under reference number 88ABW-2018-0191 and was supported by funding from the AFRL/RXAS Biological Materials and Processing Research Team via contract to B.S.S. from UES, Inc. (prime contract FA8650-15-D5405 task 0001 agreement S-111-016-001).

We acknowledge the sequencing services provided by the Oklahoma Medical Research Foundation and the Air Force Research Laboratory.

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